Does Bioavailability Limit Biodegradation?

A Comparison of Hydrocarbon Biodegradation and Desorption Rates in Aged Soils

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Two DOE-NPTO Funded Projects

Nancy Comstock, Program Manager

- Environmentally Acceptable Endpoints: The Influence of Soil Characteristics and Molecular Hydrocarbon Properties on Bioavailability and Toxicity in Aged Soils

- The Development of Innovative and Cost-Effective Methods for the Assessment of Bioavailability Limitations During Bioremediation of Aged Petroleum Hydrocarbon Contaminated Soils

First Project: Research Objectives

- How is the bioavailability of aged hydrocarbons affected by soil properties?
- How does aging affect bioavailability?
- Is incomplete PAH biodegradation caused by bioavailability limitations or microbial factors?
- What is the residual toxicity of bioremediated soils?
- Is the leaching of BTEX from crude oil contaminated soils affected by soil properties or aging?

Soil Property Data

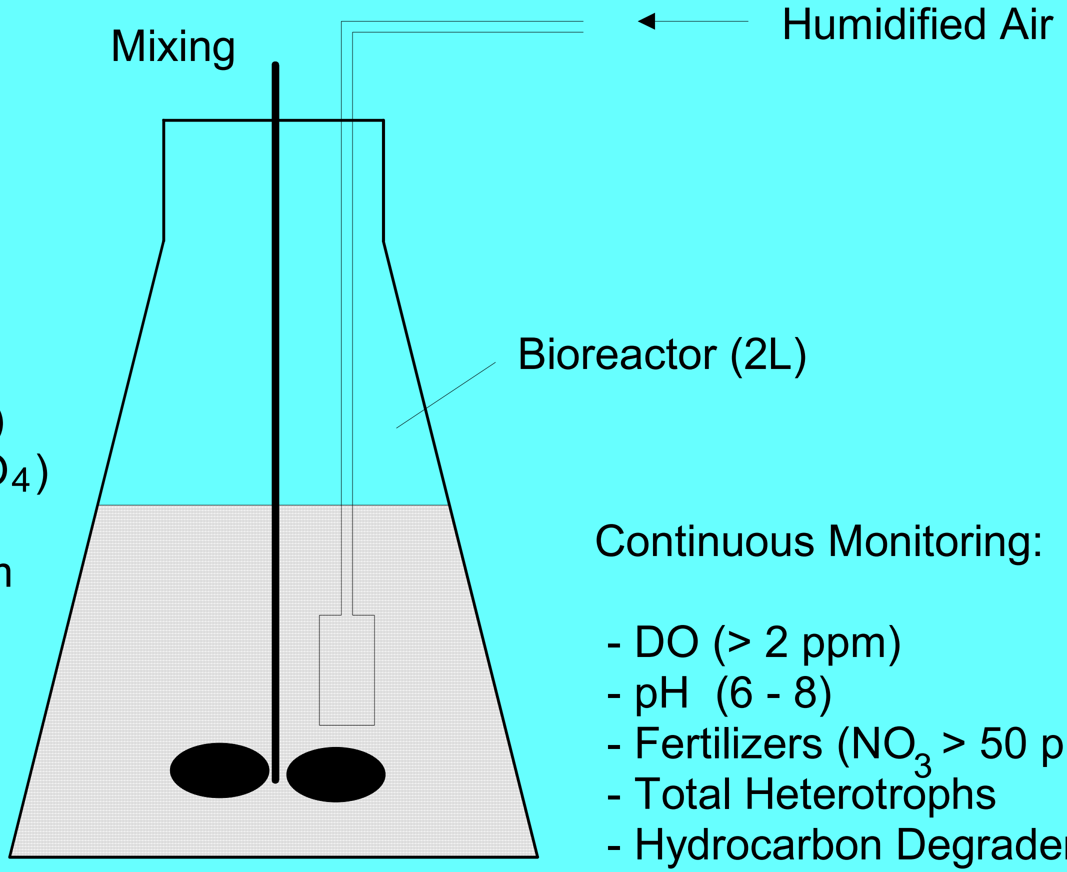
Soil / Model Solid	Organic Carbon [% dry wt]	Particle Diameter [mm]	Surface Area (BET) [m^2/g]	Surface Area (Hg Poro) [m^2/g]	Pore Volume [cm^3/g]
Quartz Sand (Fine)	0.04	0.30 - 0.42	1.2924	13.8354	0.0022
Quartz Sand (Coarse)	0.09	1.00 - 1.68	1.7075	7.90240	0.0029
Kaolinite Clay	0.07	NA	16.197	36.5921	0.1095
Montmorrilonite Clay	0.10	NA	27.544	31.4865	0.0853
Peat (Canadian)	36.2	52% < 0.05	0.7134	17.7757	0.0066
60 A Silica Gel	0.09	0.25 - 0.50	478.42	265.426	0.8084
150 A Silica Gel	0.05	0.25 - 0.50	308.00	376.224	1.1383
Richland Loam	0.30	35% < 0.05	11.965	32.0157	0.0266
Navy Field Soil	0.25	16% < 0.05	0.5755	15.1213	0.0130
Glass Beads	NA	1.00	0.0014	0.00143	0

- **All soils were spiked with crude oil (5% or 10% wt)**
- **All soils were aged in closed jars for 27 months**

Slurry Bioremediation

Soil / Water Slurry:

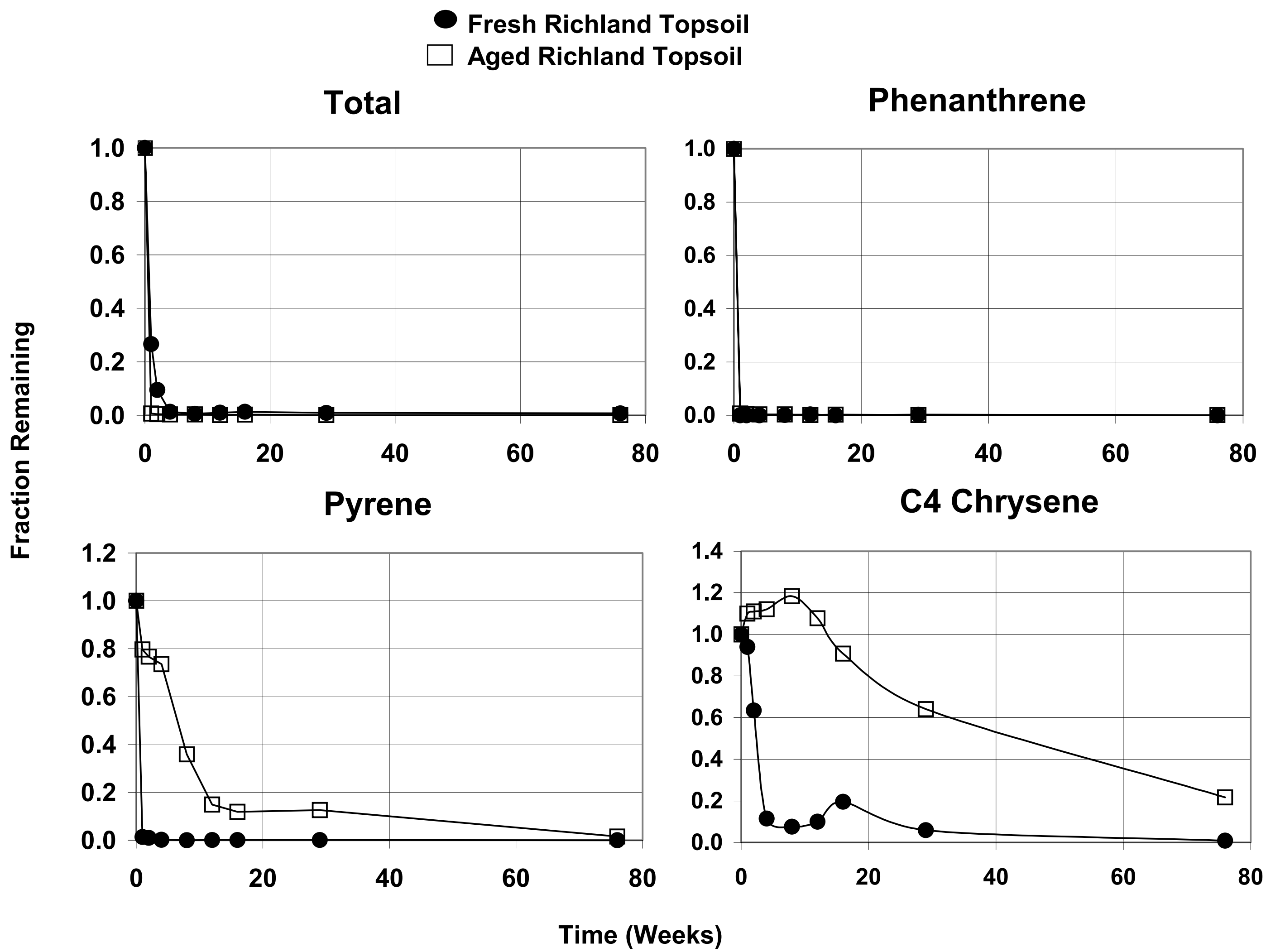
- Aged / Spiked Soil
- Distilled Water
- Dispersion Agent ($CaSO_4$)
- Fertilizer (C/N/P = 100/1/0.2)
  (NH_4NO_3, KH_2PO_4, K_2HPO_4)
- pH = 7.0
- Crude Oil Degrader Inoculum



Continuous Monitoring:

- DO (> 2 ppm)
- pH (6 - 8)
- Fertilizers (NO_3 > 50 ppm)
- Total Heterotrophs
- Hydrocarbon Degraders

Effect of Aging on Bioremediation Rates and Endpoints



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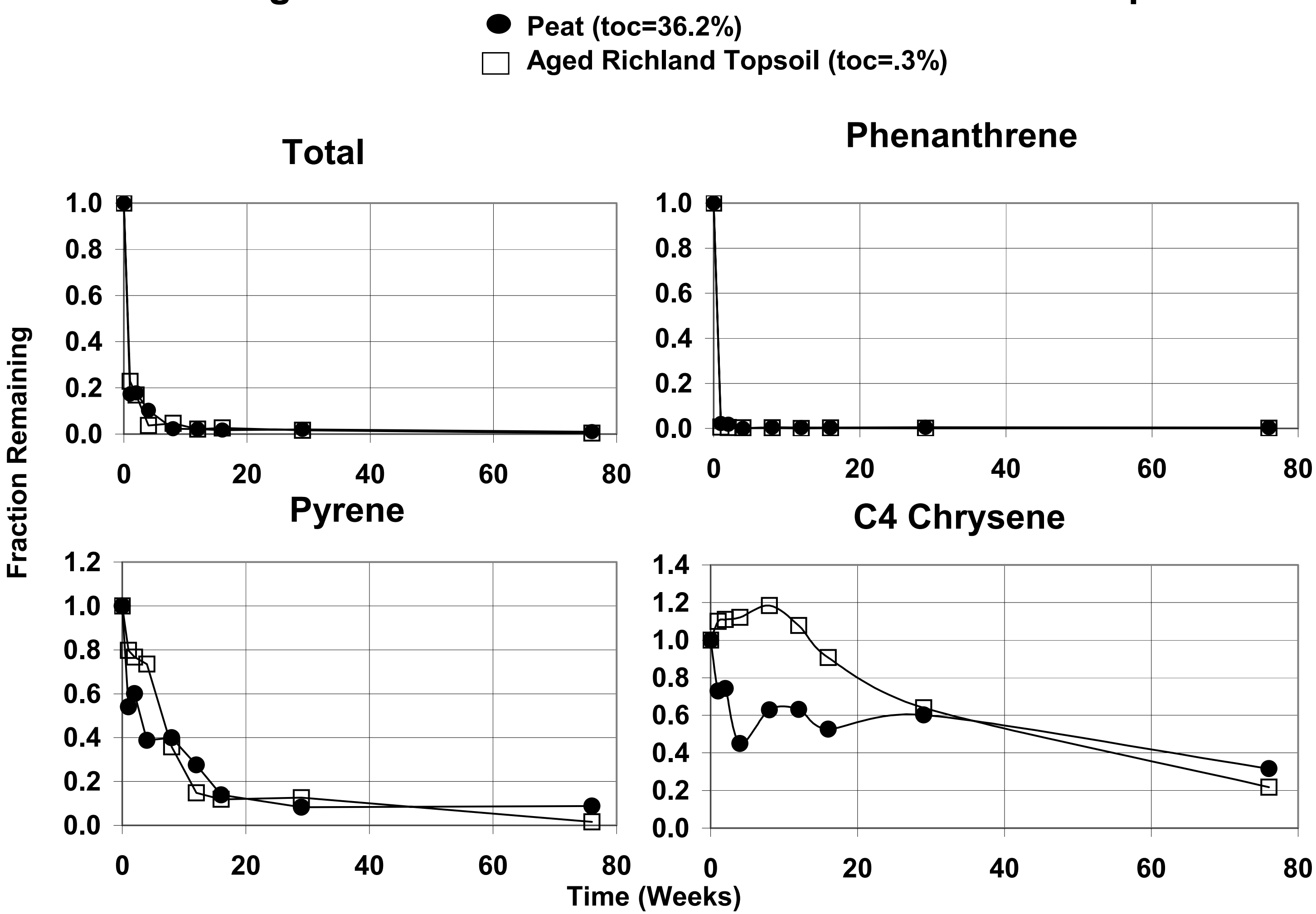
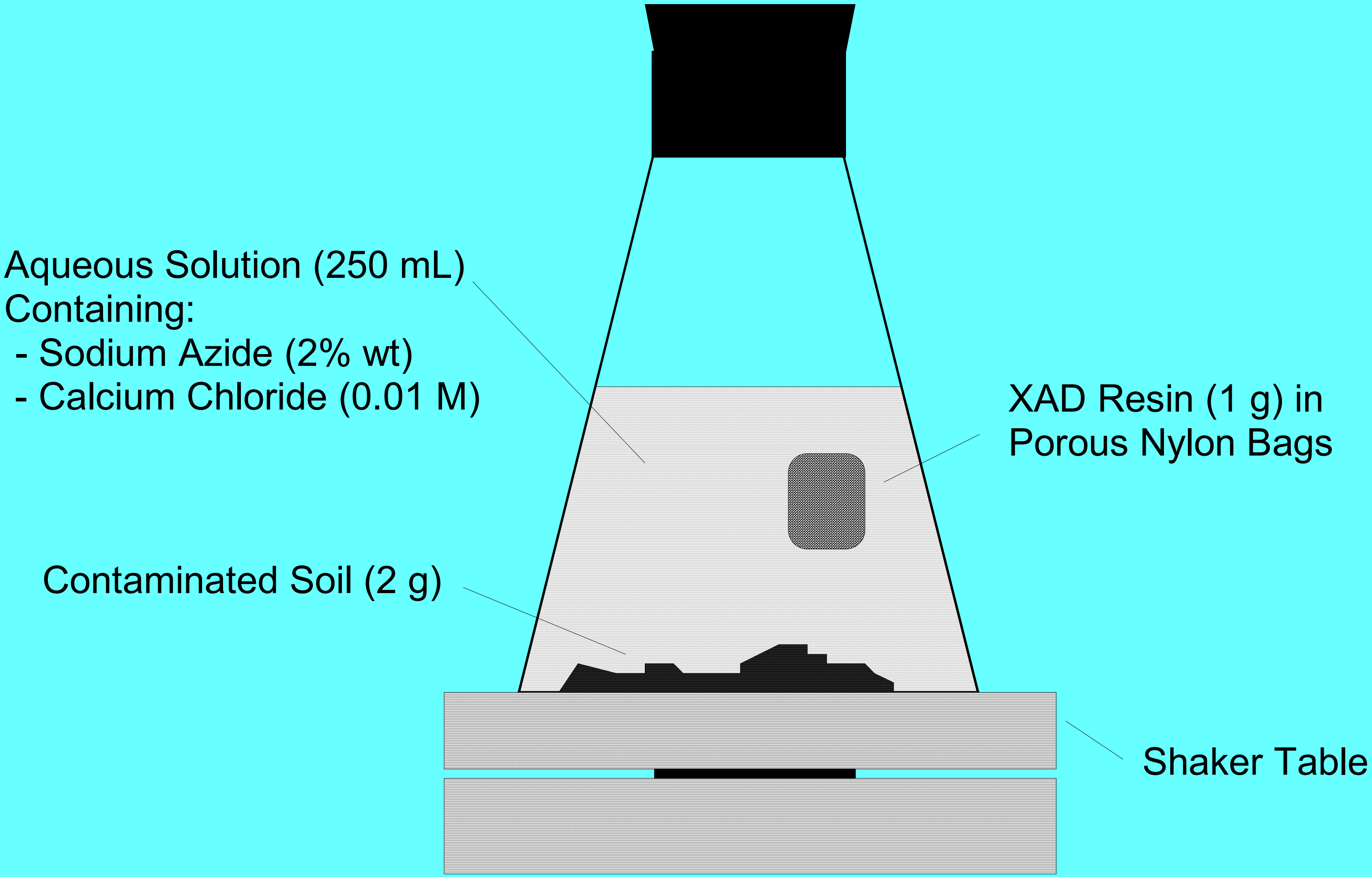
Effect of Organic Matter on Bioremediation Rates and Endpoints



Abiotic Desorption / Dissolution Kinetics



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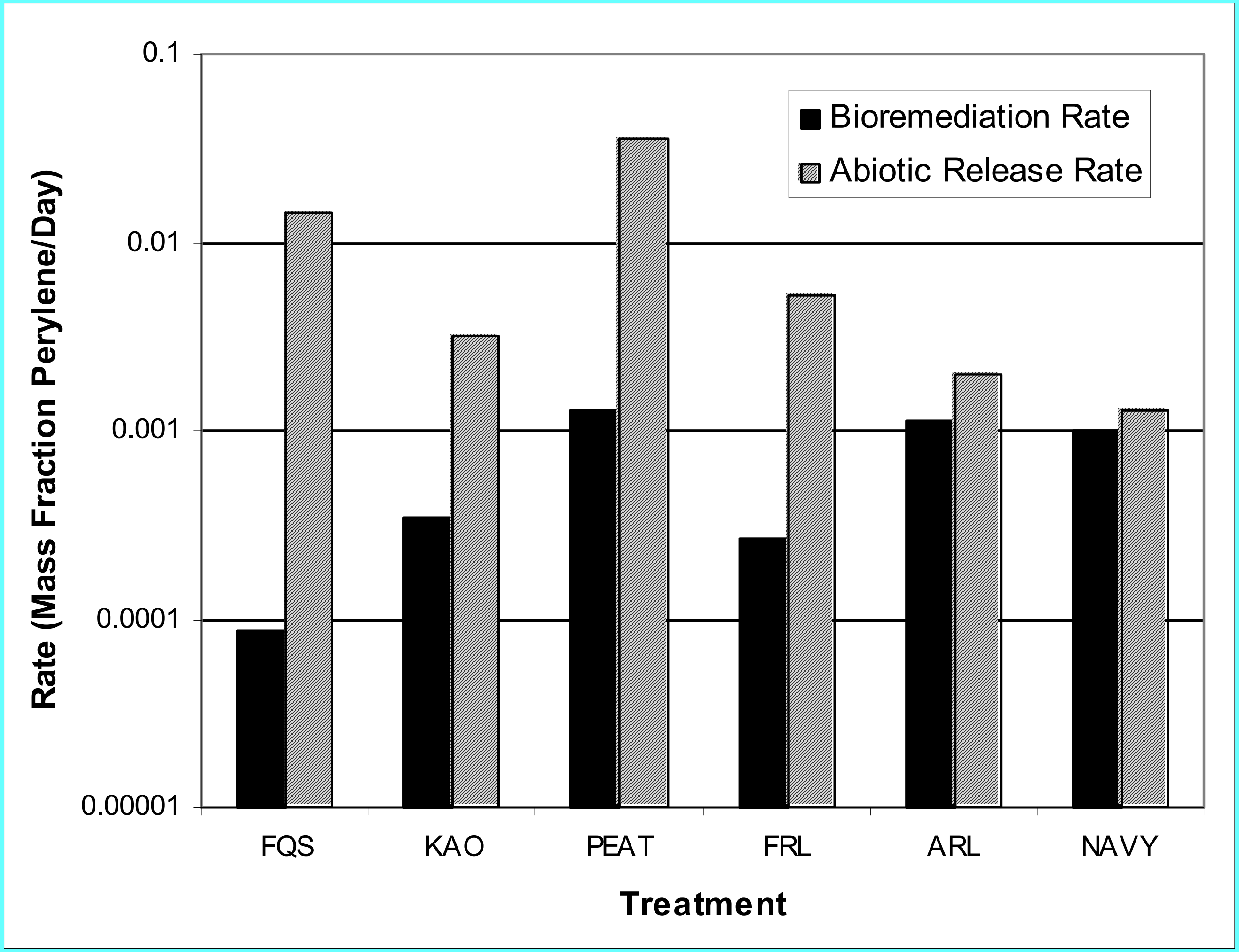
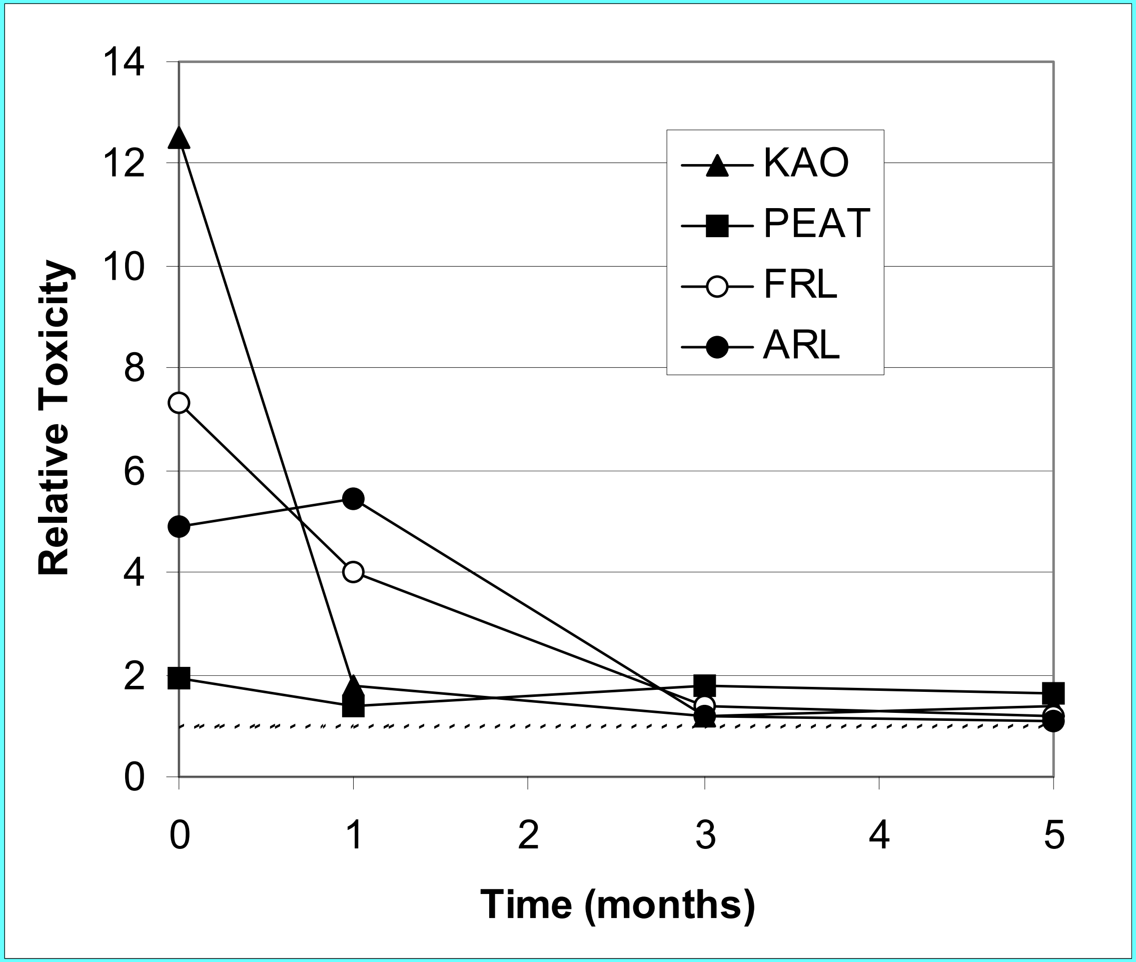
Comparison of Biodegradation and Desorption Rates at the End of Slurry Bioremediation Treatment (Week 90)



Reduction in Relative Soil Toxicity During Bioremediation



Relative toxicity = Microtox EC_{50} of clean reference soil /Microtox EC_{50} of bioreactor soil

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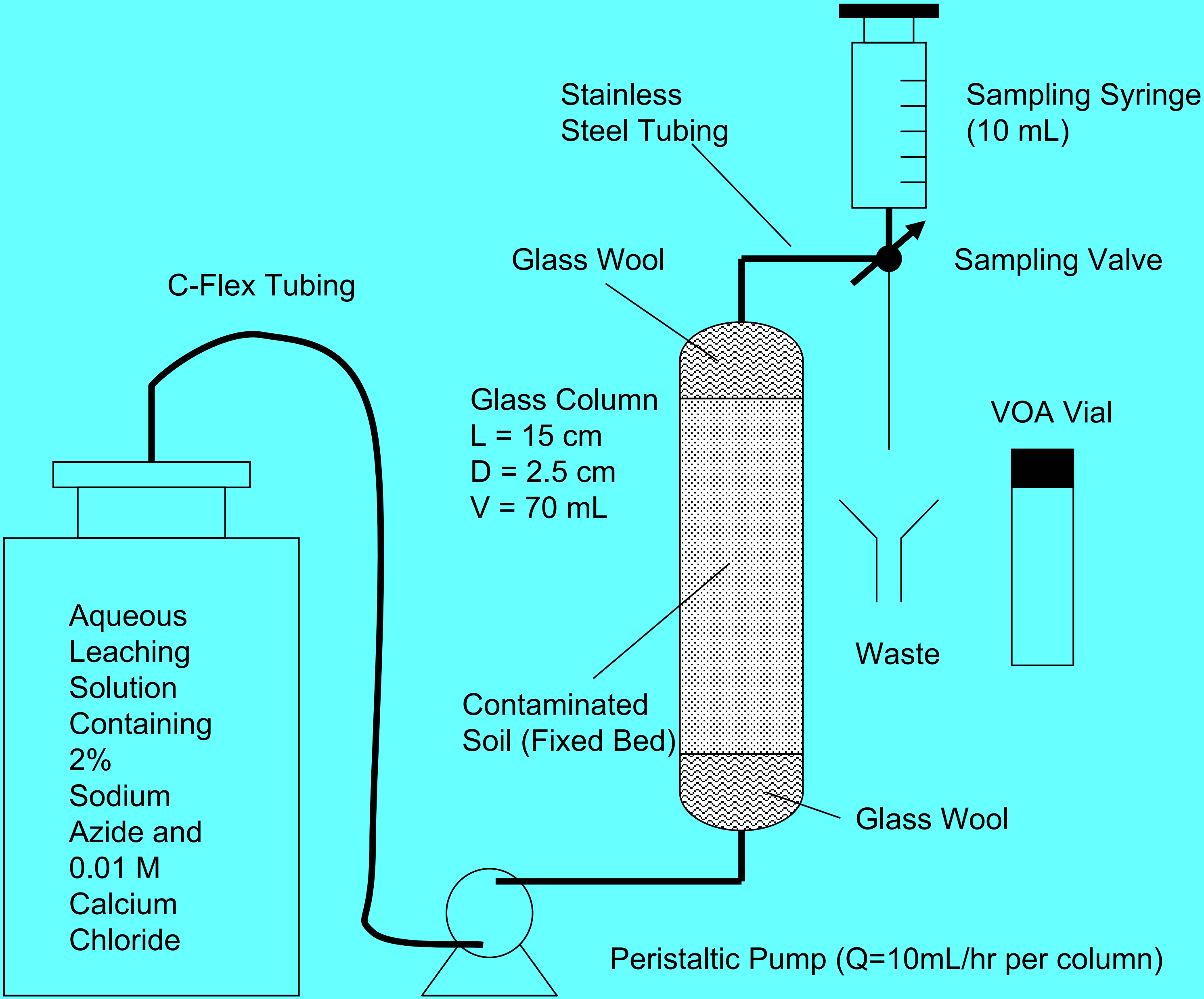
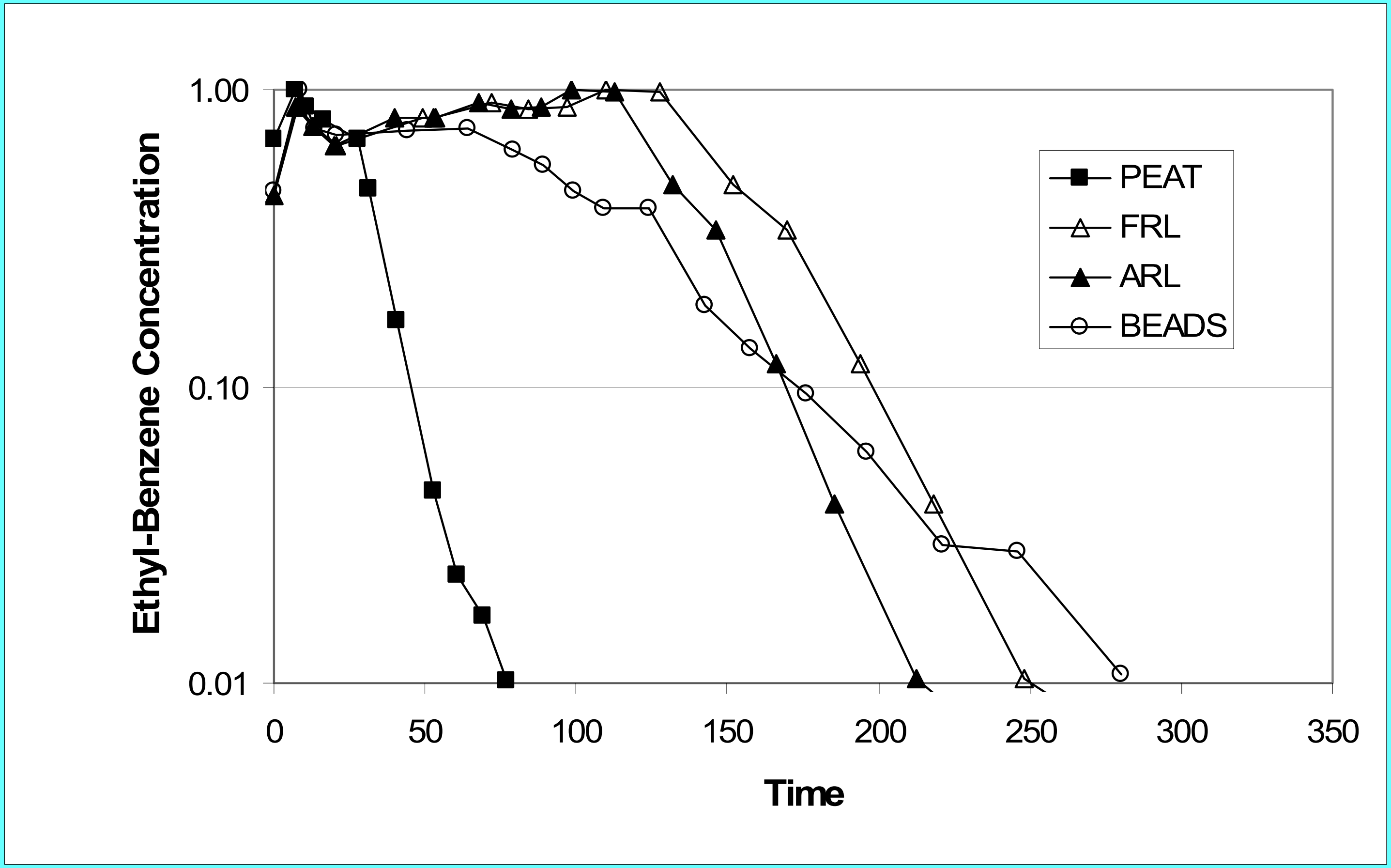

Stainless
Steel Tubing
Sampling Syringe
(10 mL)
Glass Wool
Sampling Valve
C-Flex Tubing
Glass Column
L = 15 cm
D = 2.5 cm
V = 70 mL
VOA Vial
Aqueous
Leaching
Solution
Containing
2%
Sodium
Azide and
0.01 M
Calcium
Chloride
Waste
Contaminated
Soil (Fixed Bed)
Glass Wool
Peristaltic Pump (Q=10mL/hr per column)

Figure 1

Ethyl-Benzene Concentrations as a Function of Time In the Column Effluents for Four Model Soils



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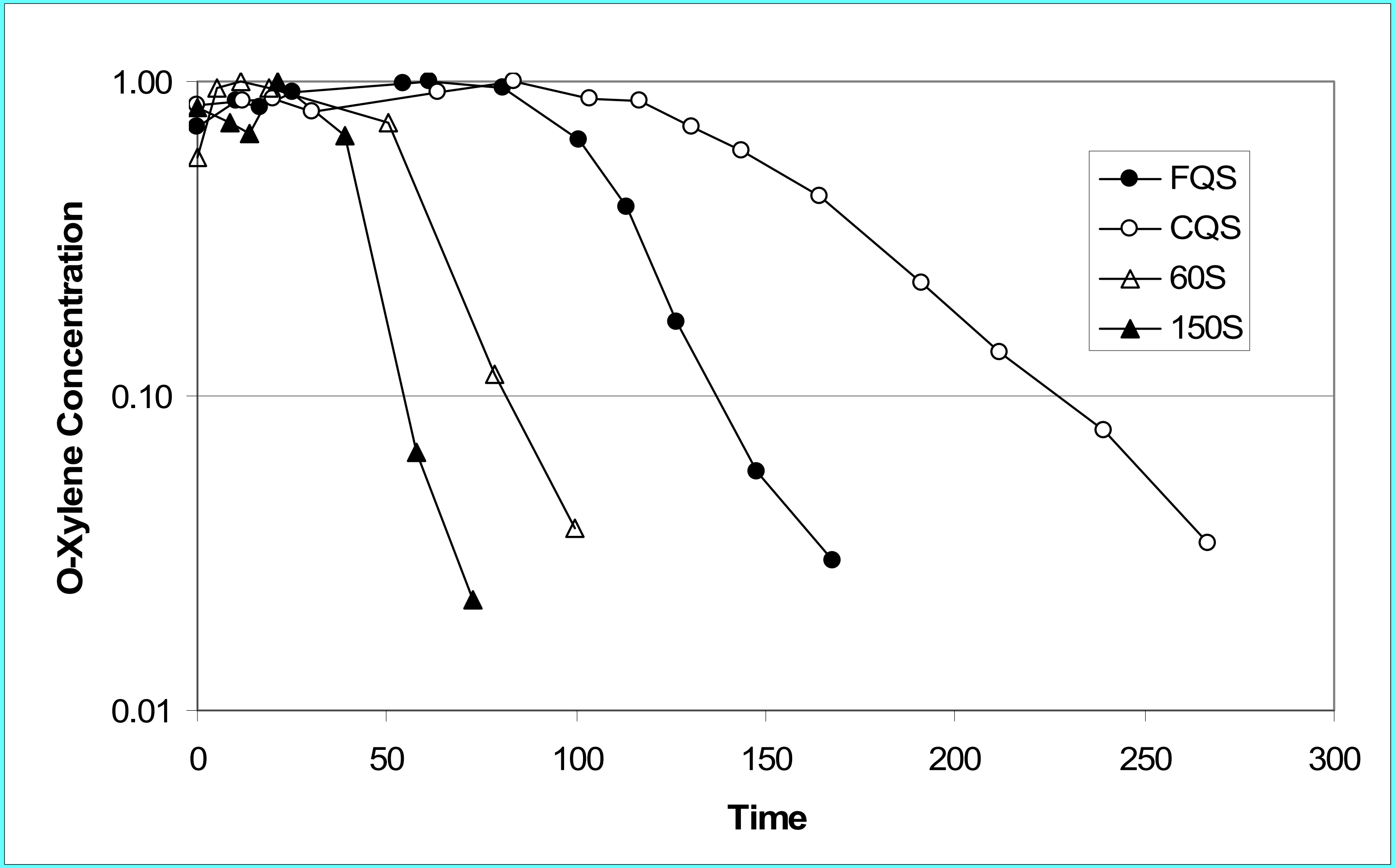
O-Xylene Concentrations as a Function of Time In the Column Effluents for Four Model Soils



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MODELING OF FIXED BED LEACHING

The dissolution of compound 'i' from a multicomponent NAPL with one-dimensional flow can be described by:

$$\frac{\partial C_i}{\partial t} = \frac{1}{Pe}\frac{\partial^2 C_i}{\partial z^2} - \frac{\partial C_i}{\partial z}$$

where:

C_i	dimensionless leachate concentration of compound 'i'
Pe	Peclet number (=vL/D), a measure of dispersion
z	dimensionless direction of flow
t	dimensionless time, given as:

$$t = \frac{Q}{M_o \cdot K_i^{o\text{-}L}} \cdot t_m$$

where:

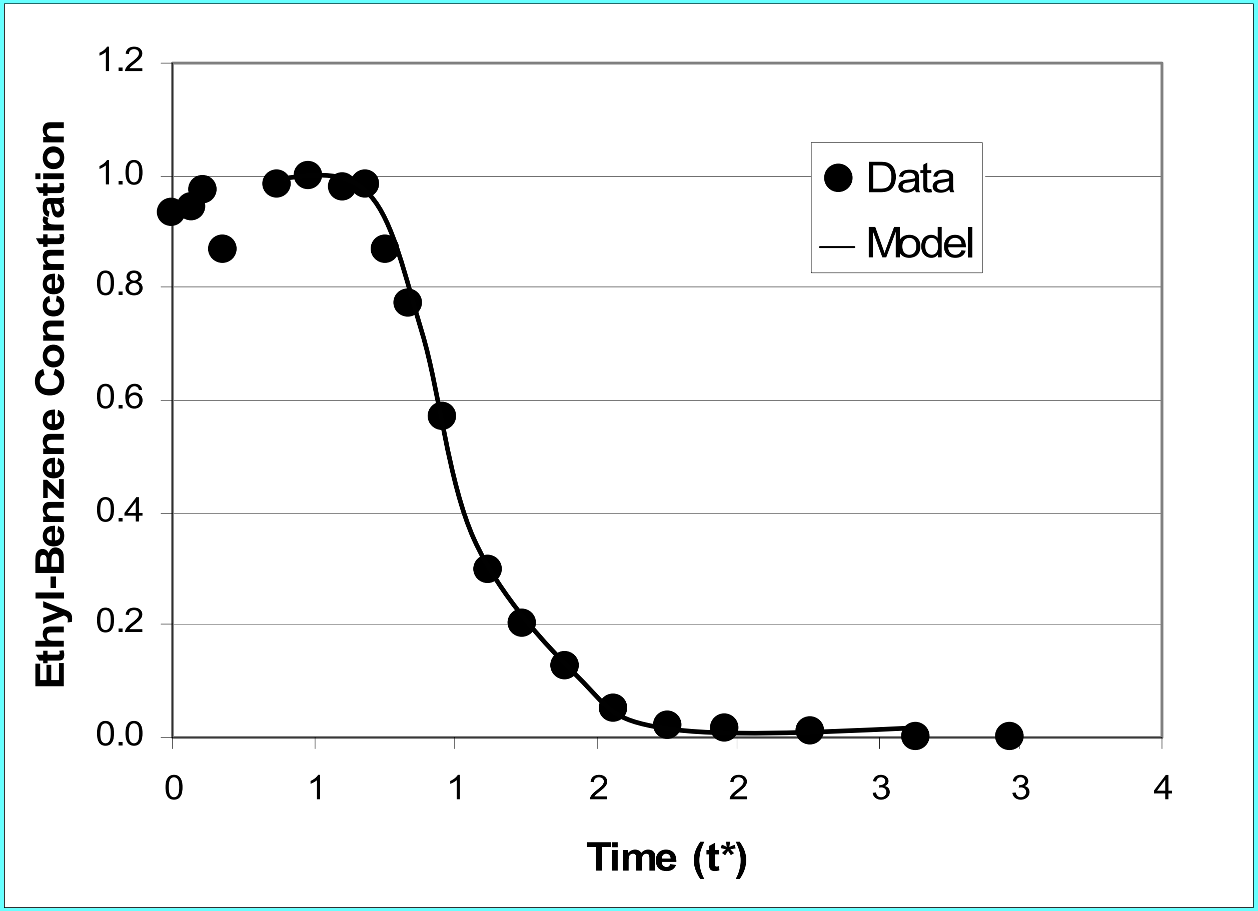
Q	leaching flow rate (L/d)
M_o	mass of oil in column (kg)
$K_i^{o\text{-}L}$	equilibrium oil-leachate partition coefficient for compound 'i' (L/kg)
t_m	measured time (d)

The solution to the one-dimensional flow equation for the column effluent concentration for compound 'i' is:

$$C_i^* = 1 - \frac{1}{2}\operatorname{erfc}\left(\sqrt{\frac{Pe}{4t^*}}(1-t^*)\right) - \frac{1}{2}e^{Pe}\operatorname{erfc}\left(\sqrt{\frac{Pe}{4t^*}}(1+t^*)\right)$$

where erfc is the complimentary error-function.

Model Fitting of Experiment Leaching Data by Adjusting the Oil-Leachate Partitioning Coefficient



Model Fits for Oil-Leachate Partition Coefficients (Kol)

Column# / Soil	Benzene	Toluene	E-Benzene	M&P-Xylene	O-Xylene
1. Fine Quartz Sand	60	300	800	800	800
2. Coarse Quartz Sand	100	300	1200	1300	1100
3. Kaolinite Clay	100	500	2000	1500	2000
4. Montmorrillonite Clay	1000	2200	4000	4000	4000
5. Canadian Peat Moss	400	800	2500	2600	2600
6. 60 Silica Gel	200	500	1500	1900	2000
7. 150 Silica Gel	330	500	2000	2000	1750
8. Fresh Richland Loam	150	400	1400	1400	1300
9. Aged Richland Loam	NA	450	1300	1300	1200
10. Glass Beads	NA	300	1000	1400	1200
Average (all columns)	292	625	1770	1820	1795
Average (w/o col.# 4)	191	450	1522	1578	1550
Rixey et al. (1999)					
Fixed Bed K^{ol}	180	450	1450	1600	NA
Batch Extraction K^{ol}	190	440	1600	1800	NA
Mackay et al. (1992) K^{ow}	135	490	1349	1585	1414

First Project: Conclusions

- The rate and extent of biodegradation is not significantly affected by soil properties, including OM content.
- The effects of aging appear more pronounced with increasing MW of the respective PAHs.
- Incomplete PAH biodegradation appears to be caused by microbial factors instead of bioavailability limitations.
- Bioremediation effectively detoxifies the soil.
- Leaching of BTEX from aged crude oil contaminated soils is not affected by soil properties but is governed by the equilibrium dissolution of these HCs from the crude oil NAPL into the aqueous phase.

Second Project: Research Objectives

- Determine whether hydrocarbon biodegradation in aged soil is mass-transfer rate-limited or reaction rate-limited?
- Stated more simply: Is it limited by bioavailability or bugs?
- Compare biodegradation and desorption rates of specific alkanes and PAHs at different time points during slurry bioremediation treatment.

SELECTION OF SOILS

Soil Type	Contamination	Aging Time
Sandy Loam	Minas Crude (5% wt)	21 months
Field Soil (Gas Plant)	Coal Tar (5% wt)	> 10 years
Beach Sand (Navy)	Bunker C (0.25% wt)	> 5 years
Belhaven (20% OM)	Mixture of 16 HC	51 months
Sassafras (10% Clay)	Mixture of 16 HC	51 months
Cullera (42% Clay)	Mixture of 16 HC	51 months

Mixture of Spiked Model Hydrocarbons

Compound Name	Conc. [mg/kg]	$\log K_{ow}$	Compound Name	Conc. [mg/kg]	$\log K_{ow}$
Nonane (C_9)	611	5.56	Pristane	1341	11.39
Decane (C_{10})	640	6.10	Naphthalene	595	3.37
Dodecane (C_{12})	798	7.18	Phenanthrene	972	4.57
Tetradecane (C_{14})	878	8.26	Pyrene	1114	5.18
Hexadecane (C_{16})	1050	9.34	Benzo(a)pyrene	1296	6.04
Eicosane (C_{20})	1534	11.5	1-Octadecene	1039	9.96
Docosane (C_{22})	1715	12.58	Cis-Decalin	507	5.25
Tetracosane (C_{24})	1982	13.66	Me-Cyc-Hexane	411	3.88

Slurry Bioremediation

Soil / Water Slurry:

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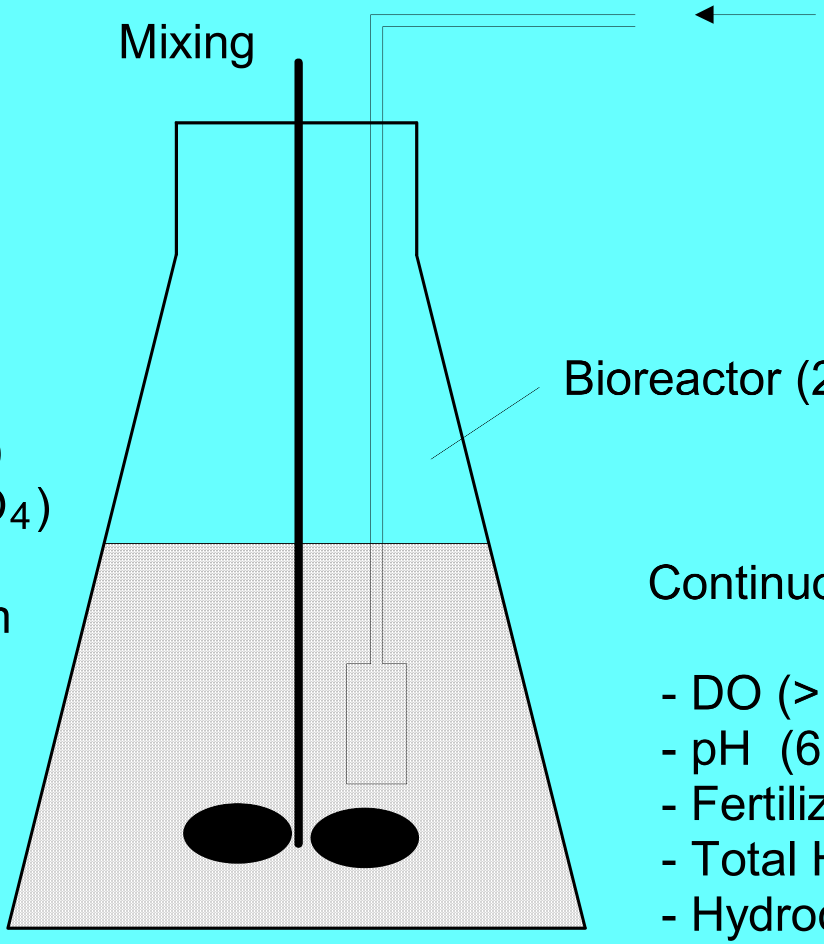
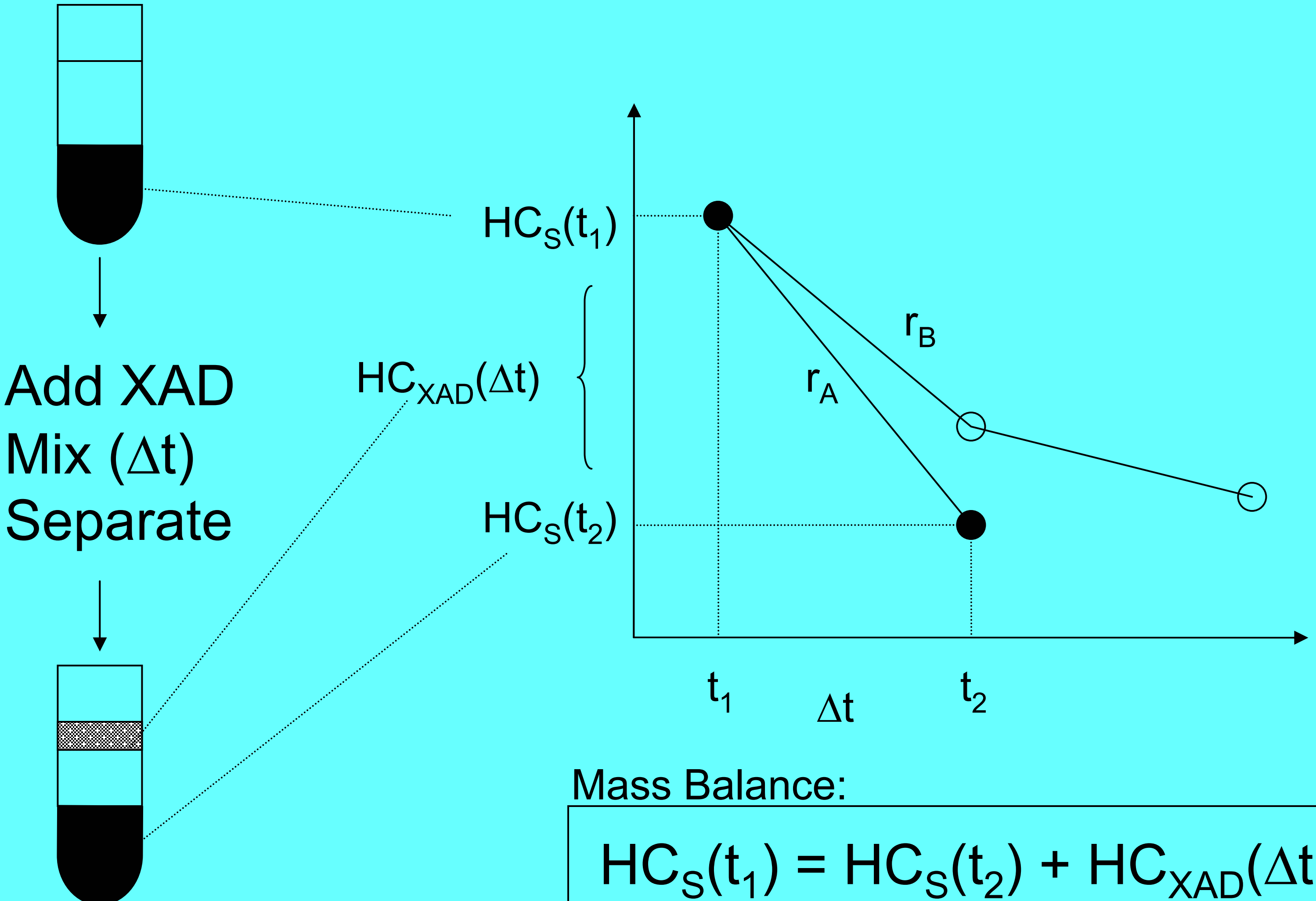
- Distilled Water
- Dispersion Agent ($CaSO_4$)
- Fertilizer (C/N/P = 100/1/0.2)
  (NH_4NO_3, KH_2PO_4, K_2HPO_4)
- pH = 7.0
- Crude Oil Degrader Inoculum



Continuous Monitoring:

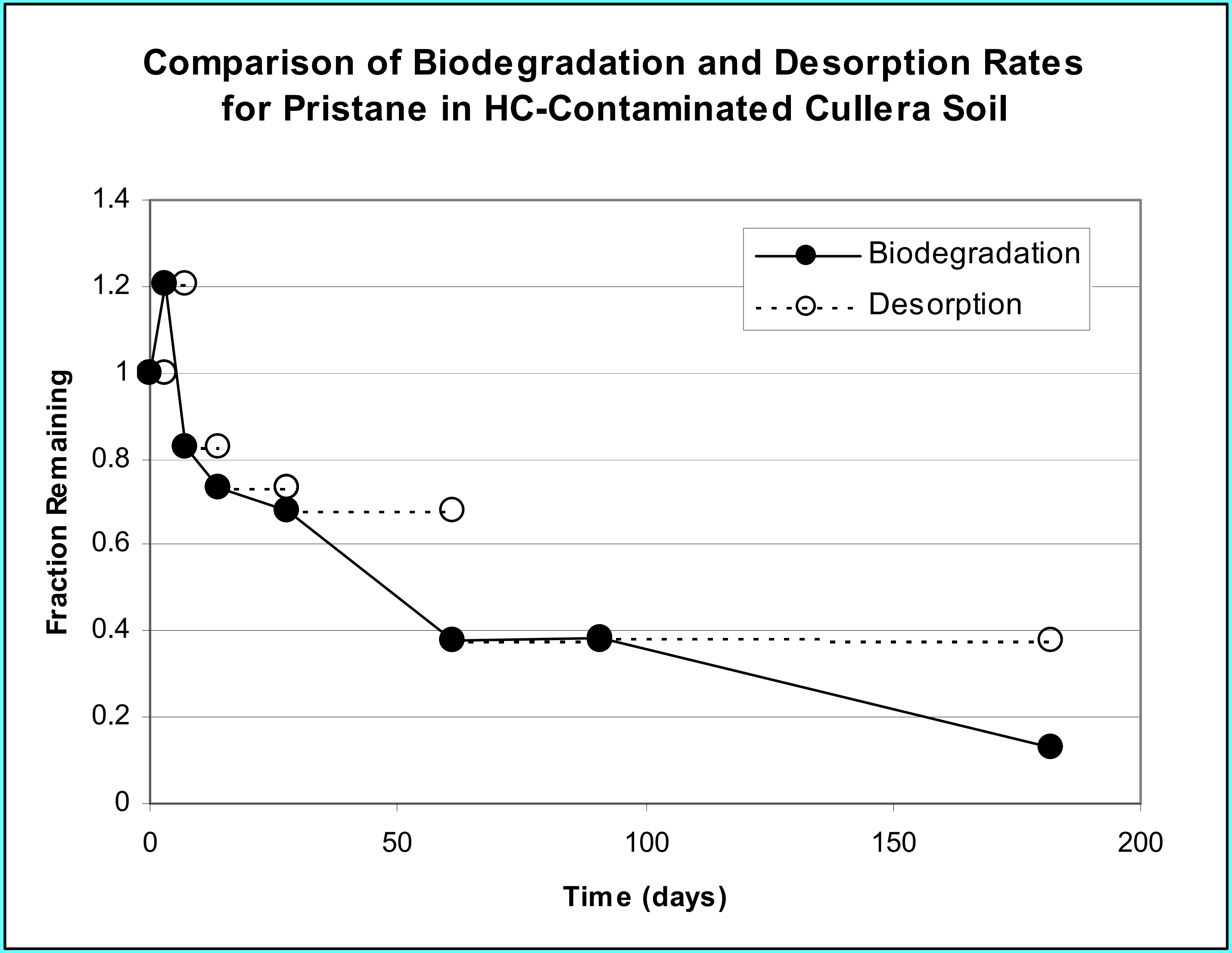
- DO (> 2 ppm)
- pH (6 - 8)
- Fertilizers (NO_3 > 50 ppm)
- Total Heterotrophs
- Hydrocarbon Degraders

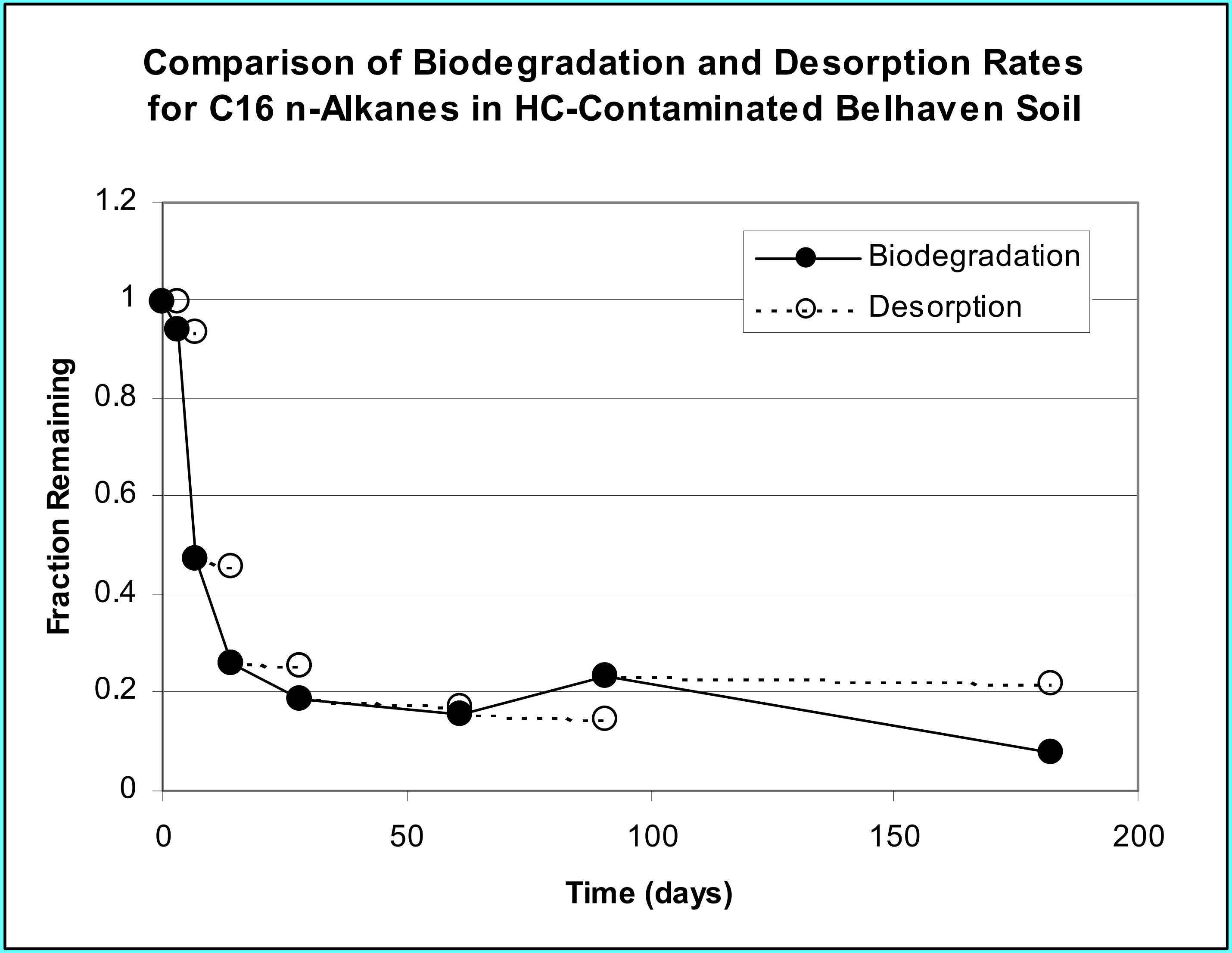
Abiotic Release Rate Assay



Mass Balance:

$$HC_S(t_1) = HC_S(t_2) + HC_{XAD}(\Delta t)$$


Comparison of Biodegradation and Desorption Rates
for Pristane in HC-Contaminated Cullera Soil
Biodegradation
Desorption
Fraction Remaining
Time (days)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
50
100
150
200



Comparison of Biodegradation and Desorption Rates for C16 n-Alkanes in HC-Contaminated Belhaven Soil
1.2
1
0.8
0.6
0.4
0.2
0
Fraction Remaining
0
50
100
150
200
Time (days)
Biodegradation
Desorption

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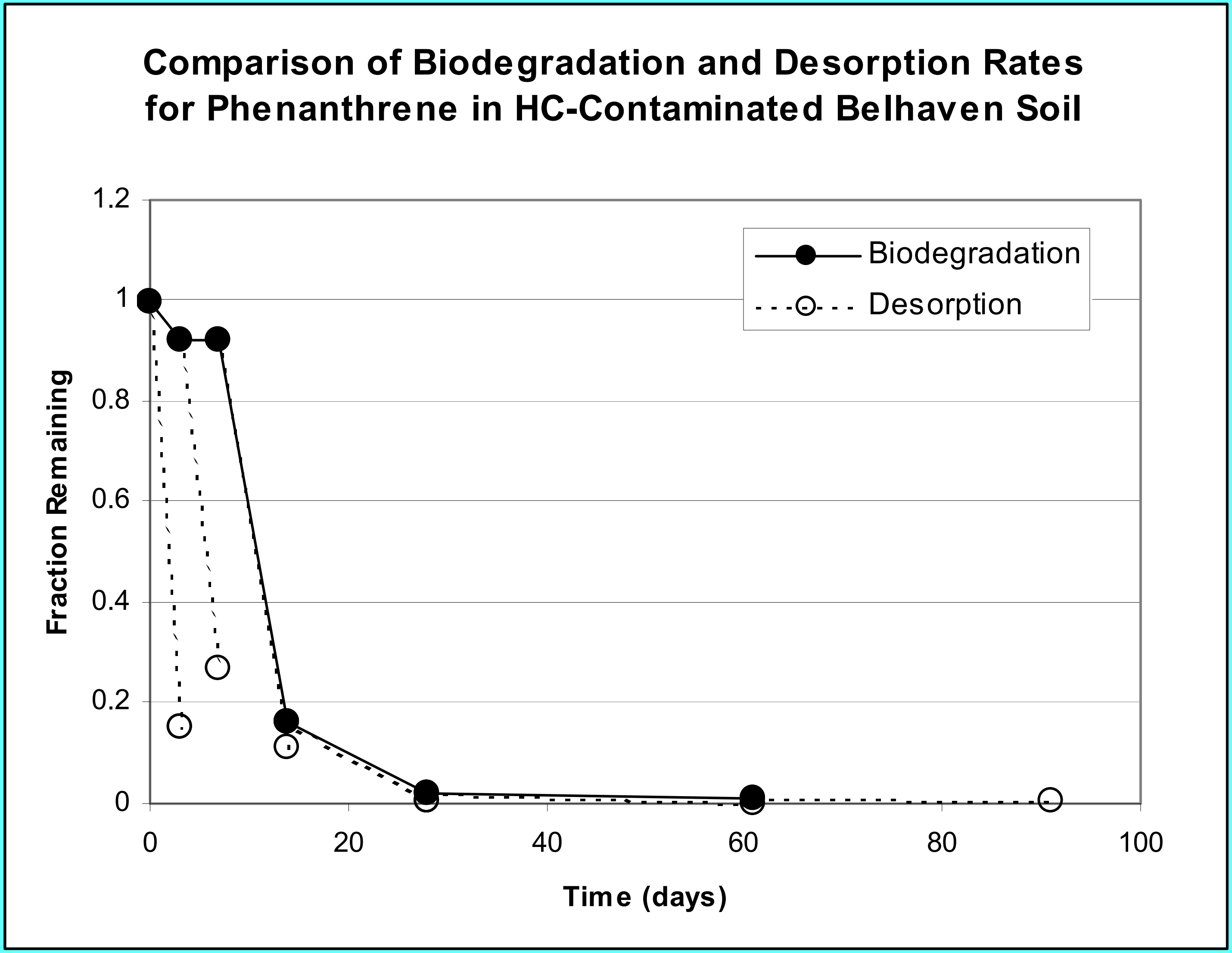
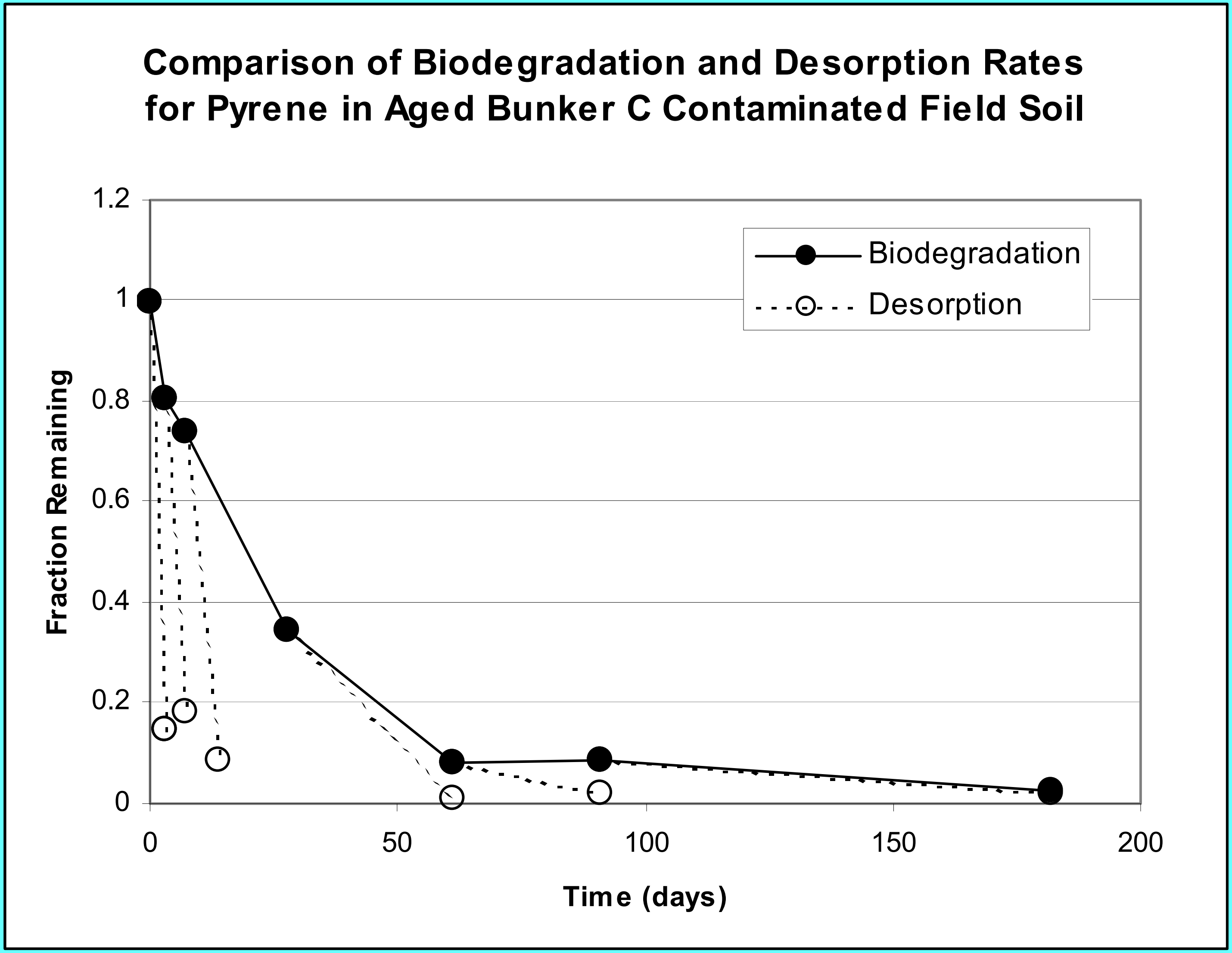
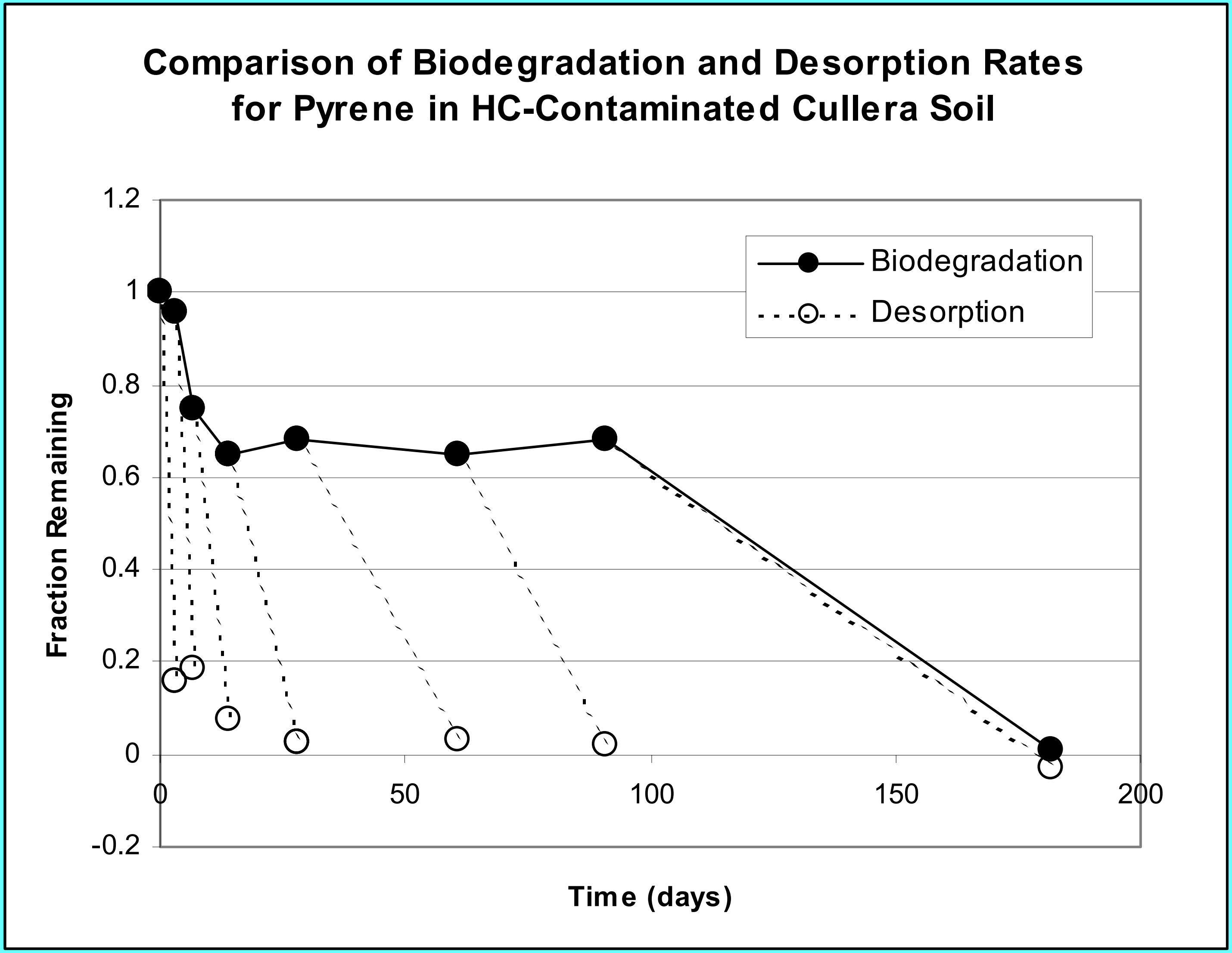
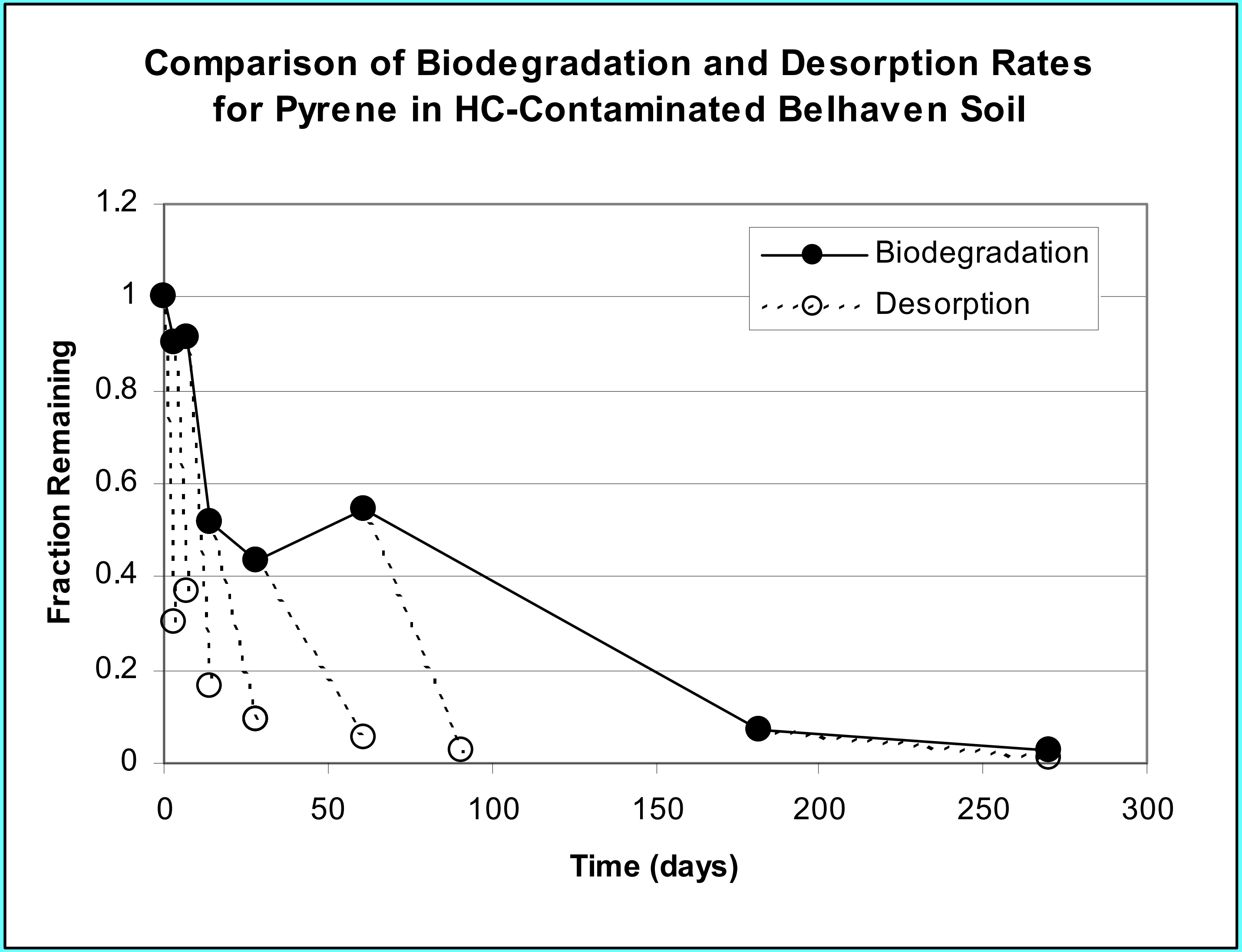
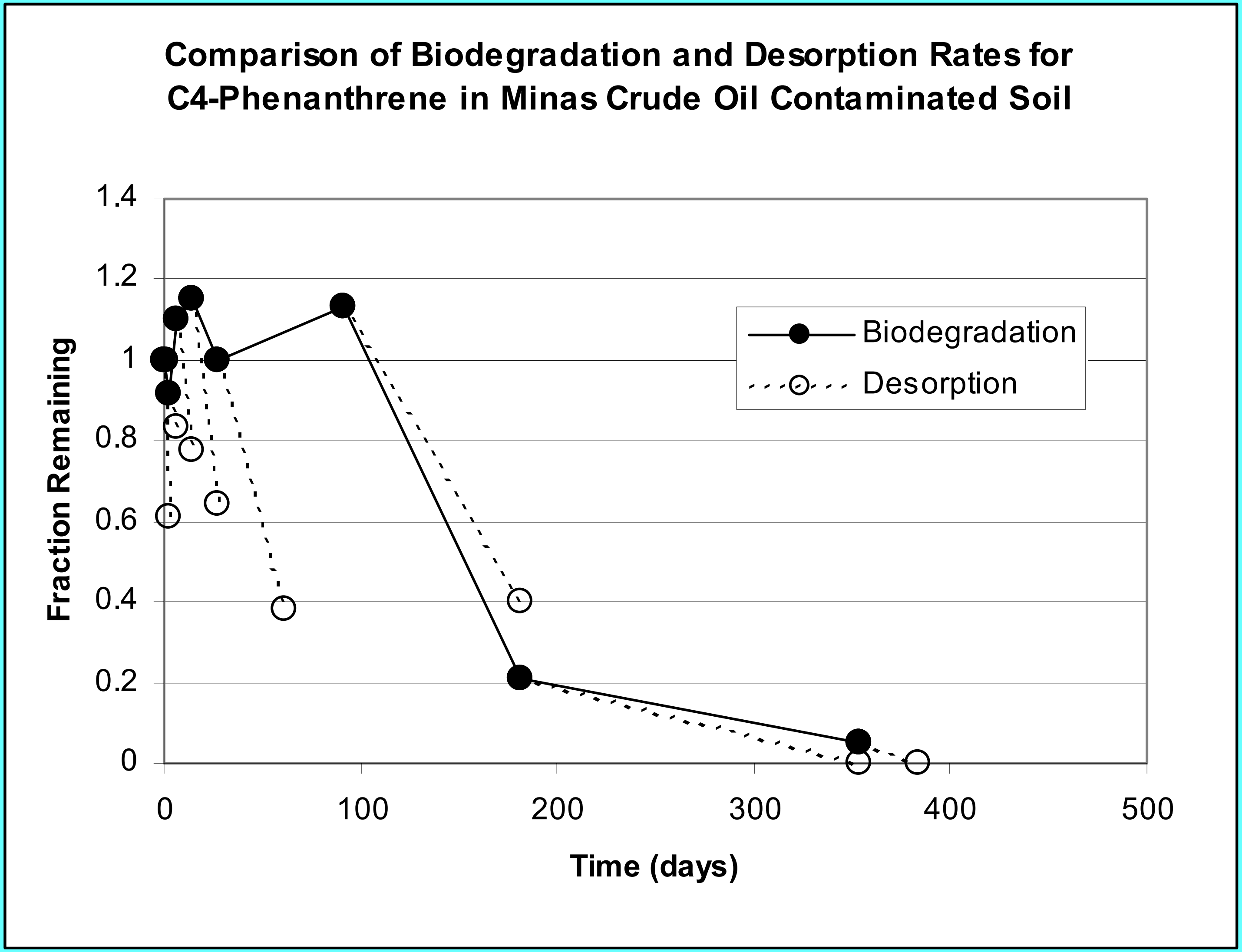

Comparison of Biodegradation and Desorption Rates
for Phenanthrene in HC-Contaminated Belhaven Soil
1.2
1
0.8
0.6
0.4
0.2
0
Fraction Remaining
0
20
40
60
80
100
Time (days)
Biodegradation
Desorption


Comparison of Biodegradation and Desorption Rates
for Pyrene in Aged Bunker C Contaminated Field Soil
1.2
1
0.8
0.6
0.4
0.2
0
Fraction Remaining
0
50
100
150
200
Time (days)
Biodegradation
Desorption


Comparison of Biodegradation and Desorption Rates
for Pyrene in HC-Contaminated Cullera Soil
Biodegradation
Desorption
Fraction Remaining
Time (days)
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
50
100
150
200



Comparison of Biodegradation and Desorption Rates
for Pyrene in HC-Contaminated Belhaven Soil
Fraction Remaining
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
Time (days)
Biodegradation
Desorption



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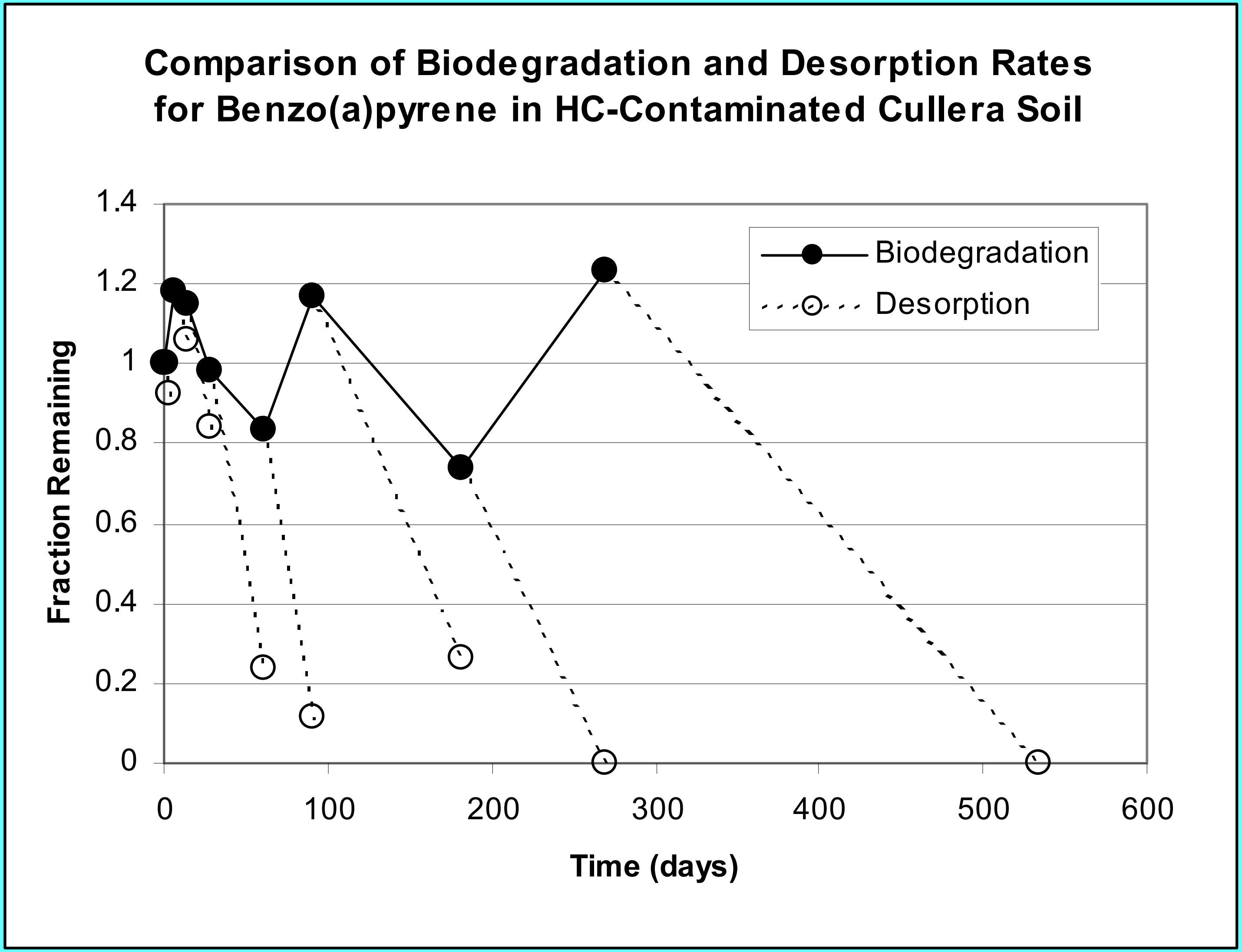
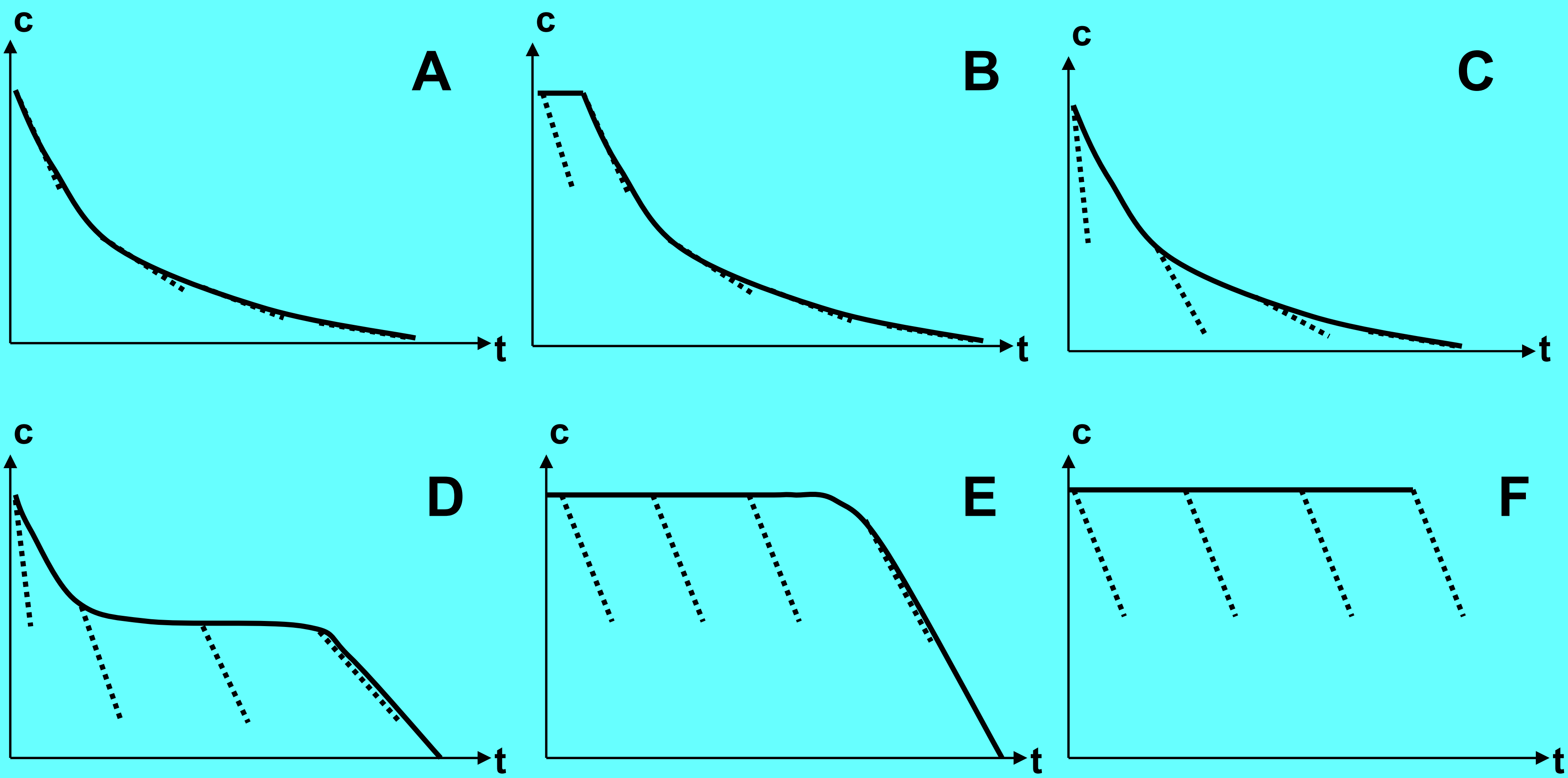

Comparison of Biodegradation and Desorption Rates
for Benzo(a)pyrene in HC-Contaminated Cullera Soil
Biodegradation
Desorption
Fraction Remaining
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
Time (days)

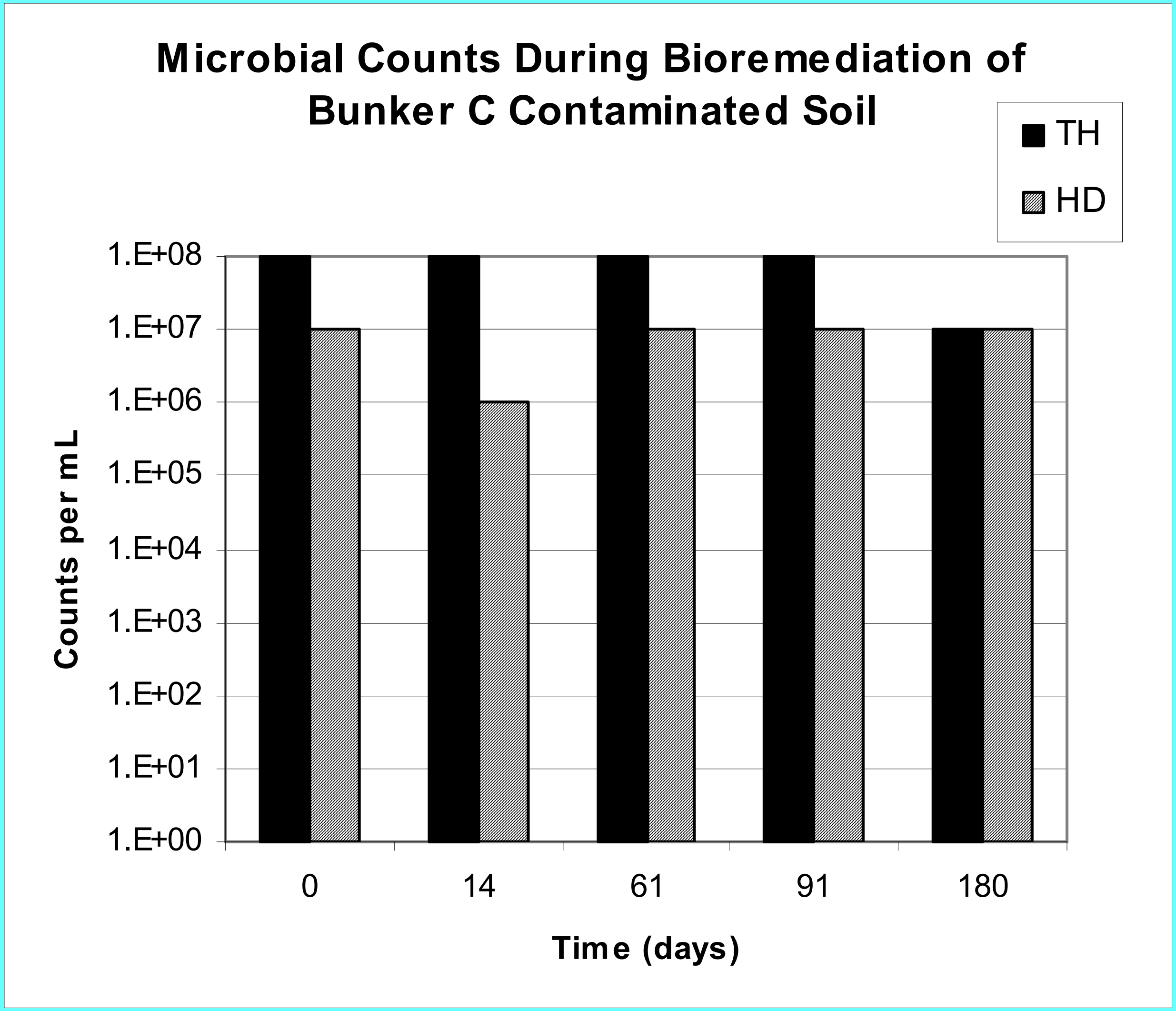
Summary of Observed Scenarios

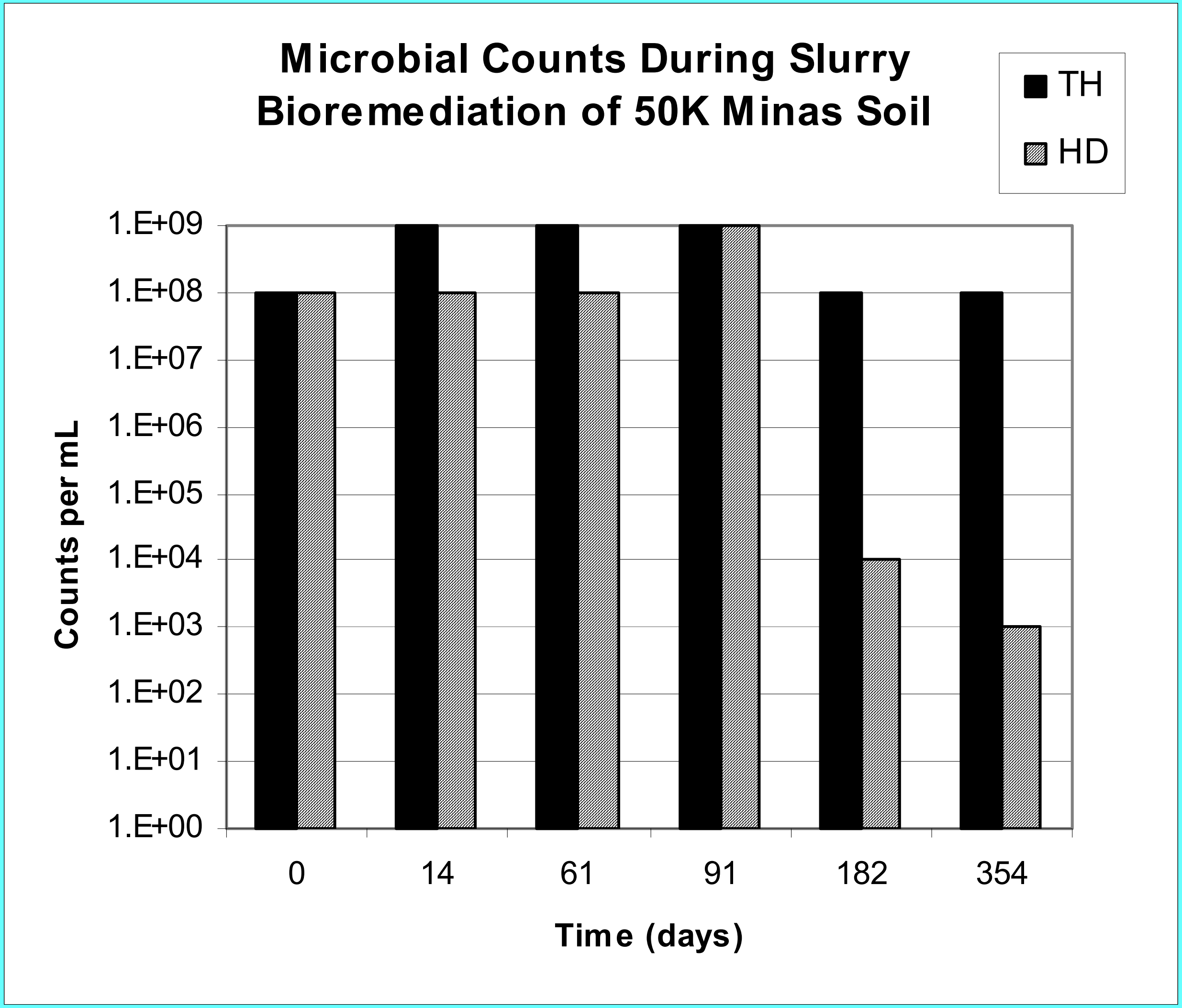


THREE HYPOTHESES

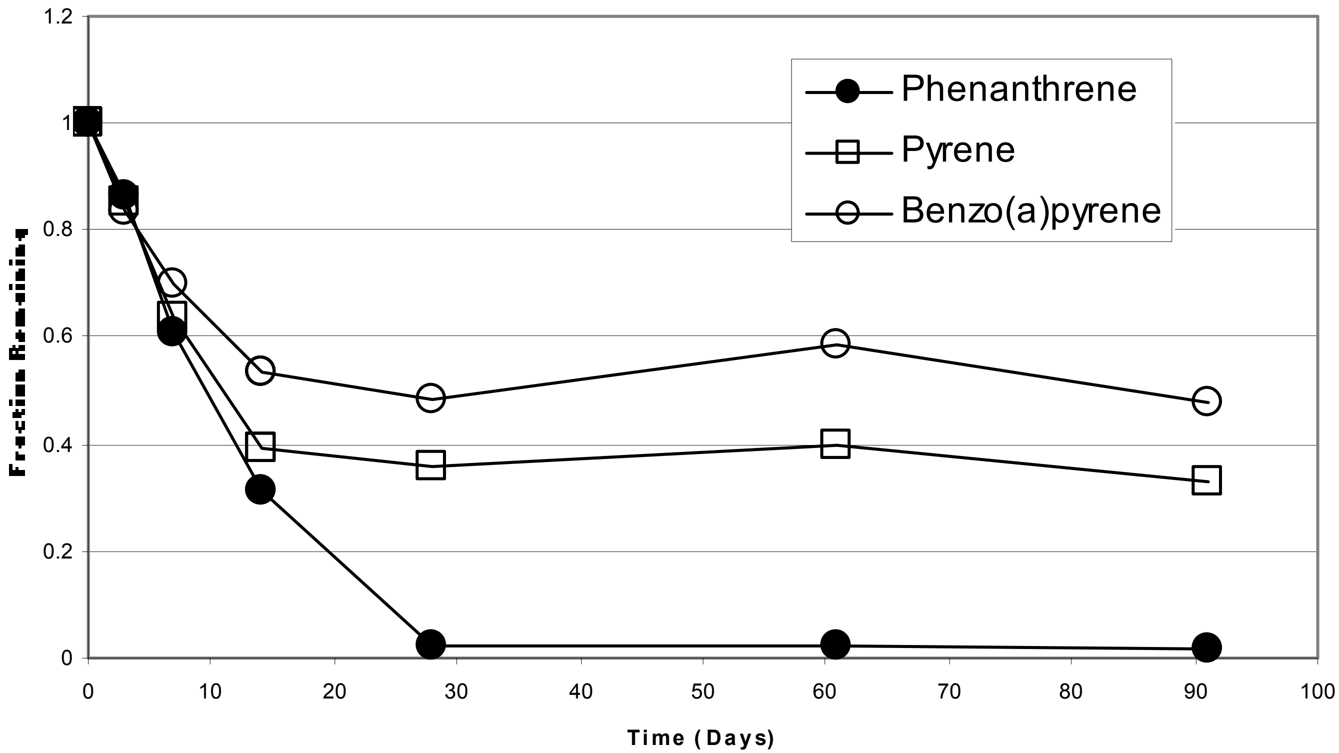
Lack of Biodegradation for "Fully Bioavailable" PAHs Could be Caused By:

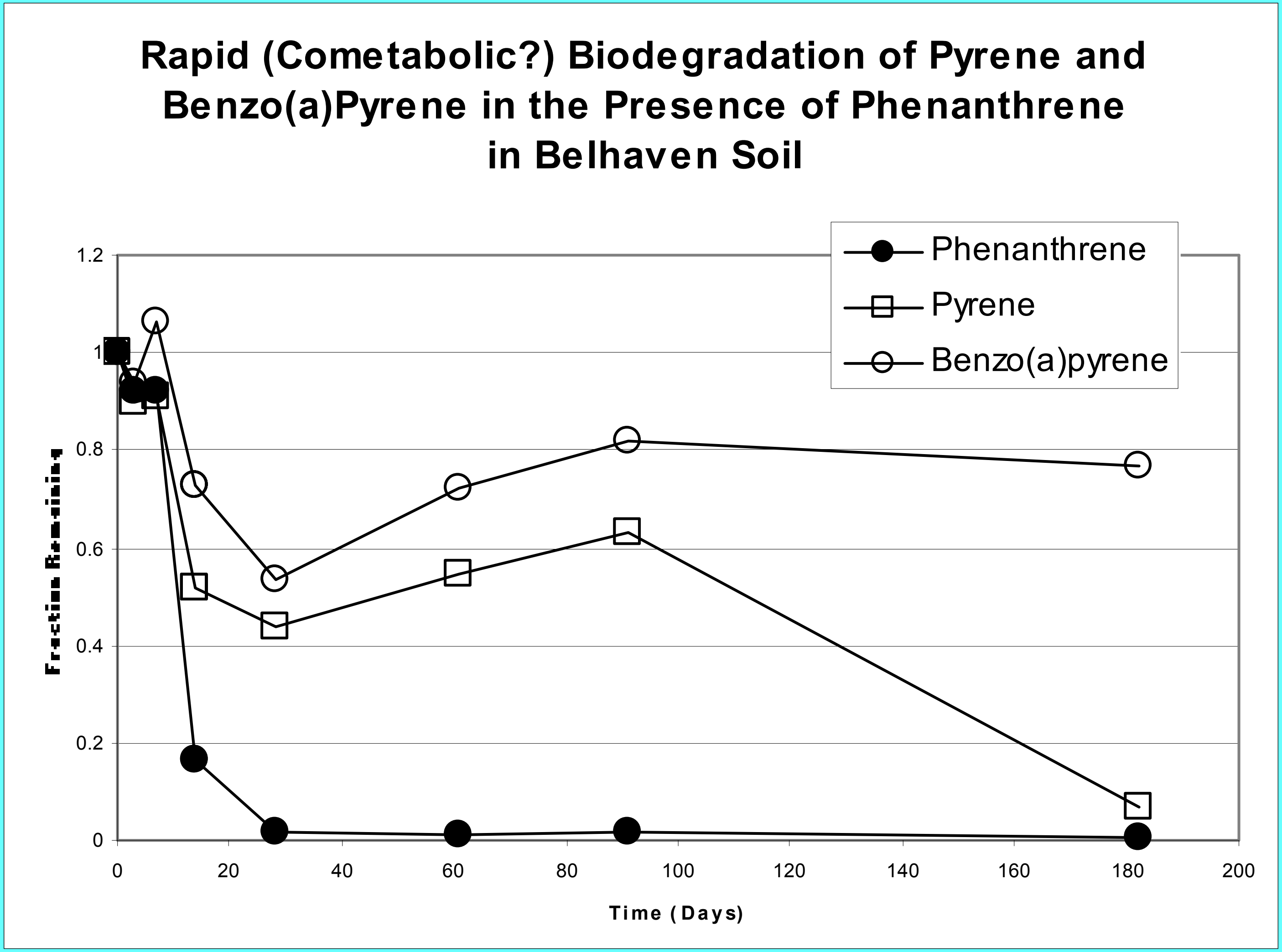
- **Inhibition Effects**
- **Absence of Specific Hydrocarbon Degraders**
- **Lack of Cometabolism**


Microbial Counts During Bioremediation of Bunker C Contaminated Soil
TH
HD
Counts per mL
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
0
14
61
91
180
Time (days)


Microbial Counts During Slurry Bioremediation of 50K Minas Soil
TH
HD
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Counts per mL
0
14
61
91
182
354
Time (days)

Rapid (Cometabolic?) Biodegradation of Pyrene and Benzo(a)Pyrene in the Presence of Phenanthrene in Sassafras Soil




Rapid (Cometabolic?) Biodegradation of Pyrene and Benzo(a)Pyrene in the Presence of Phenanthrene in Belhaven Soil
Phenanthrene
Pyrene
Benzo(a)pyrene
Fraction Remaining
Time (Days)
0
0.2
0.4
0.6
0.8
1
1.2
20
40
60
80
100
120
140
160
180
200

Second Project: Conclusions

- Alkanes Biodegrade Rapidly Despite the Fact that They Do Not Desorb from the Soil.
- If PAH Biodegradation Occurs, the Biodegradation Kinetics May or May Not Be Limited by the Abiotic Release Rate.
- Lack of PAH Biodegradation is Not Caused by Bioavailability Limitations and Therefore must be Entirely Due to Microbial Factors such as Inhibition Effects, Absence of Hydrocarbon Degraders, or Lack of Cometabolism.
- Residual PAHs May Pose Higher Risk Than Previously Thought.

Publications

1. Huesemann, M.H., T. Hausmann, and T. Fortman, "Comparison of PAH biodegradation and desorption kinetics during bioremediation of aged petroleum hydrocarbon contaminated soils", In: Case Studies in the Remediation of Chlorinated and Recalcitrant Compounds, G.B. Wickramanayake, A.R. Gavaskar, J.T. Gibbs, and J.L. Means, Editors, Battelle Press, Columbus, OH, 2000, pp. 181-188.
2. Huesemann, M.H., T. Hausmann, and T. Fortman, "Assessment of mass-transfer limitations during slurry bioremediation of PAHs and alkanes in aged soils", In: Bioremediation of Energetics, Phenolics, and Polycyclic Aromatic Hydrocarbons, V.S. Magar, G. Johnson, S.K. Ong, and A. Leeson, editors, Battelle Press, Columbus, OH, pp. 157-163, 2001.
3. Huesemann, M.H., T. Hausmann, and T. Fortman, "Microbial factors rather than bioavailability limit the rate and extent of PAH biodegradation in aged crude oil contaminated model soils", *Bioremediation Journal*, **6(4):**321-336, 2002.
4. Huesemann, M.H., T.S. Hausmann, and T.J. Fortman, "Assessment of bioavailability limitations during slurry bioremediation of petroleum hydrocarbons in aged soils", *Environmental Toxicology and Chemistry*, **22(12):**2853-2860, 2003.
5. Huesemann, M.H., T.S. Hausmann, and T.J. Fortman, "Biodegradation of hopane prevents use as conservative biomarker during bioremediation of PAHs in petroleum contaminated soils", *Bioremediation Journal*, **7(2):**111-117, 2003.
6. Huesemann, M.H., T.S. Hausmann, and T.J. Fortman. 2004. "Reduction of toxicity and PAH bioaccumulation potential during bioremediation of petroleum-contaminated soils." In: V.S. Magar and M.E. Kelley (Eds.), *In Situ and On-Site Bioremediation—2003.* Proceedings of the Seventh International In Situ and On-Site Bioremediation Symposium (Orlando, FL; June 2003). Paper J-05. ISBN 1-57477-139-6, published on compact disk by Battelle Press, Columbus, OH.
7. Huesemann, M.H., T.S. Hausmann, and T.J. Fortman, "Does bioavailability limit biodegradability? A comparison of hydrocarbon biodegradation and desorption rates in aged soils", *Biodegradation*, **15(4):**261-274, 2004.
8. Huesemann, M.H., "Biodegradation and bioremediation of petroleum pollutants in soil", Chapter 2 in *Soil Biology - Applied Bioremediation and Phytoremediation*, A. Singh and O.P. Ward, editors, Volume 1, Springer Verlag, Berlin Heidelberg, pp. 13-34, 2004.
9. Huesemann, M.H., T.S. Hausmann, and T.J. Fortman, "Leaching of BTEX from aged crude oil contaminated model soils: Experimental and modeling results", *Journal of Soil Contamination*, submitted, 2004.